recombinant GM-CSF has been trialed as a treatment for IBD, with a high incidence of adverse effects offsetting the reduction of disease activity. Auto-antibodies to GM-CSF have been detected in patients with ileal CD and βc receptor expression on granulocytes and monocytes is repressed in patients with IBD, particularly UC patients, collectively suggesting that GM-CSF-βc signaling is suboptimal in IBD (Goldstein et al., 2011). How these past findings play into the role of the IL-23-GM-CSF-eosinophil axis in IBD, is unclear. Disease heterogeneity and the different roles for IL-23 that are observed in IBD subtypes are likely to be important in this respect (Kobayashi et al., 2008). However, once validated in specific subgroups (endotypes) of patients, these findings are likely to offer significant advances in IBD therapies. Anti-IL-5 therapies are already in advanced stages of clinical trials for eosinophilic disease and allergy and are readily transferrable to IBD management. Similarly, anti-IL-23 monoclonal therapies are in clinical trials for IBD and eliminating the Th17 cell-eosinophil axis may prove more effective than anti-TNF-α therapies. A more simple therapeutic approach might be inhibiting eosinophil peroxidase (EPO) with antioxidants and thus limiting the major factor inducing the lesions observed in these studies. Of note, rectal administration of vitamin E, a potent anti-oxidant, has shown some efficacy in mild-moderate ulcerative colitis (Mirbagheri et al., 2008) and ironically, inhibition of peroxidase activity by the free-radical scavenging properties of cigarette smoke might explain the reported beneficial effects of smoking in UC patients because cigarette smoke can directly inhibit EPO activity. However, human IBD is complex and there is likely to be redundancy across multiple effector pathways that operate in parallel. The findings of Griseri et al. might nonetheless lead to significant advances in our understanding of immunopathology, chronicity, and distinction of phenotypic differences in IBD subtypes. Whether these findings also extend to Th17-mediated diseases in other organs will be of considerable interest.

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Passenger Mutations Identified in the Blink of an Eye

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Passenger mutations specific to particular mouse strains can distort experimental outcomes. In this issue of *Immunity*, Vanden Berghe et al. (2015) demonstrate that passenger mutations are frequent in most genetically engineered congenic mice and persist even after extensive backcrossing.

An impressive number of genetically modified mice have been created since the techniques for gene targeting in mice were established in the 1980s by the 2007 Nobel Laureates Mario Capecchi, Oliver Smithies, and Martin Evans. To date, at least 11,000 genes have been specifically deleted or inactivated in inbred mice (Hall et al., 2009). Moreover, institutions like the International Mouse Phenotype Consortium (IMPC), the Texas A&M Institute for Genomic Medicine (TIGM), and the EUCOMMTOOL Project are engaged in generating collections of genetically engineered mutants that include genetic ablations for all of the roughly 25,000 protein-coding genes in the mouse. The comparison of congenic mice, one bearing a targeted mutation and one retaining the wild-type (WT) allele of the gene of interest, is a critical tool

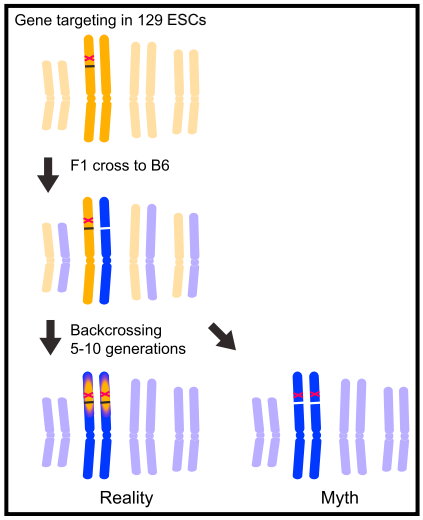

Figure 1. Myth versus Reality: Passenger Genome Mutations in Gene-Targeted Mice

(Top) Several mouse chromosome pairs in 129 ESCs are depicted in shades of orange, with a genetically modified gene of interest indicated by the red cross. An allele of a non-targeted gene that is specific to strain 129 is indicated as a black bar on the chromosome in close proximity to the targeted DNA. This allele produces a non-functional protein.

(Middle) The gene-targeted 129 ESCs are utilized to generate chimeric mice, and these chimeras are crossed to B6 mice to produce F1 descendants. These progeny bear homologous pairs of blue B6 and orange 129 chromosomes. However, the sequence of the allele of the non-targeted gene in the B6 strain (white bar) differs from that in the 129 strain and produces a functional protein.

(Bottom) After repetitive backcrossing of F1 mice to B6 mice with concomitant selection for descendants carrying the genetically modified gene, intercrosses of two heterozygotes eventually generate offspring that are homozygous for the gene-targeted mutation. The myth of this approach is that essentially all of the 129-specific genetic material has been eliminated and the B6 non-targeted allele is present in these homozygotes. In reality, backcrossing cannot fully eliminate the 129 passenger genome (orange regions of blue chromosomes), leading to potential genomic contamination with the 129-specific allele of the non-targeted gene (black bar on blue chromosome). Adapted from Lusis et al. (2007).

for revealing the biological function of that gene in vivo. Indeed, such mutants frequently serve as models for human genetic diseases, and analyses of these animals have often led to seminal scientific discoveries.

Gene-targeted mice are traditionally generated by modifying the gene of interest in embryonic stem cells (ESCs) in vitro using homologous recombination, an evolutionarily conserved DNA repair mechanism. The typical procedure involves drug-mediated selection of donor 129 ESC clones carrying the introduced genetic modification, followed by injection of these ESCs into C57BL/6 (B6) recipient blastocysts. Some of these embryos then develop into chimeric organisms composed of genetically distinct cells. Identification of chimeras that underwent germline transmission is the key to establishing a line of progeny bearing the desired mutation. ESCs of various 129 substrains have been originally chosen for production of genetically modified mice because they exhibit more efficient germline transmission and greater genomic stability in vitro than B6 ESCs (Gertsenstein et al., 2010). However, the differences in genomic background between 129 and B6 ESCs have the potential to drastically distort research outcomes. Thus, germline-transmitting chimeras are routinely backcrossed to B6 mice for ten or more generations with concomitant selection for the introduced mutation. This strategy is supposed to guarantee that the donor 129 genome is eliminated from the descendants. However, the "passenger genome," which encompasses the genomic region closely flanking the targeted locus, remains of donor origin because recombination frequency diminishes considerably near the inserted mutation (Figure 1). Previous studies have shown that genes within the donor 129 passenger genome can differ in nucleotide sequence from the corresponding allele in the recipient B6 genome (as reviewed by Lusis et al., 2007). From the researcher's viewpoint, nucleotide alterations that change the amino acid sequence of a given protein, so-called "nonsynonymous substitutions," are undesirable. Such differences can have a detrimental impact if the mutated passenger allele affects the function of the targeted gene or the experimental readout. This consequence of the use of ESCs and recipient mice of differing genetic backgrounds has long been known to scientists in the field but is often discounted. In this issue of *Immunity*, Vanden Berghe et al. (2015) provide an in-depth exploration of the insidious nature of passenger mutations and the dangers of ignoring their existence.

In their first analysis, Vanden Berghe et al. (2015) performed bioinformatics and comparative genomic studies of various inbred mouse strains using commonly available online datasets provided by the Mouse Genome Informatics (MGI, http://www.informatics.jax.org/), the Mouse Genome Project of the Wellcome Trust Sanger Institute and the IMPC (Keane et al., 2011; Yalcin et al., 2011). Their results show that at least 80% of all gene-targeted mouse strains generated to date are based on donor 129 ESCs. Comparison of the complete 129 genome with the reference B6 sequence revealed that 1,084 genes exhibit insertions or deletions of DNA nucleotides (indels) or single nucleotide polymorphisms (SNPs) resulting in nonsynonymous substitutions. Of these alterations, 13% are nonsense and 63% are frameshift mutations. An additional 24% of 129 genomic mutations alter splice donor and acceptor sites, which could lead to the expression of splice variants with activities or functions quite different from those of the protein encoded by the reference B6 allele. Strikingly, therefore, almost all genetically engineered congenic mouse strains created thus far carry strain 129 passenger mutations that could potentially compromise phenotype determination. To illustrate the magnitude of the problem, if the size of the genomic region flanking the targeted gene is fixed to only 1 centimorgan (1cM), there is still a probability of 0.91 that this region remains of 129 origin even after 10 backcrosses to B6 mice. Remarkably, 69% of all genetically modified congenic mice carry 1 to 20 passenger mutations within the 1 cM genomic region flanking an introduced genetic modification.

Vanden Berghe et al. (2015) then translated their in silico findings to live mice using the well-established model of lethal lipopolysaccharide (LPS) challenge. Macrophages infected with a sufficient number of LPS-bearing *Escherichia coli* synthesize lethally high amounts of interleukin-1β, and caspase-11 (*Casp11*) is essential for this production. B6 mice that bear a functional *Casp11* gene therefore rapidly succumb to injection of large amounts of LPS. In contrast, 129 mice express a *Casp11* allele with a 5 base pair (bp) deletion in the exon 7 splice acceptor site that leads to an aberrant splicing product, a frameshift, and a premature stop codon. This inactivating mutation

of *Casp11* protects 129 mice from LPS-induced death, providing a textbook illustration of the potential impact of passenger mutations (Kayagaki et al., 2011). In their study, Vanden Berghe et al. (2015) identified a total of 294 genes within a 10 cM genomic region flanking the *Casp11* gene. They then identified 86 mouse models in which one of these 294 genes had been targeted using 129 ESCs. However, to date, only two of these models, Casp1-deficient mice and Birc2-deficient mice, have been demonstrated to carry the 129-specific inactivating mutation of *Casp11* (Kayagaki et al., 2011; Kenneth et al., 2012). Vanden Berghe et al. (2015) therefore investigated the matrix metalloproteinase *Mmp13* gene that resides within a 1 cM DNA region flanking the *Casp11* gene. Despite more than 10 backcrosses to the B6 strain, the examined *Mpp13*-ablated mice still carried the 129-specific *Casp11* mutation. Thus, these *Mpp13* null mice were completely protected against a lethal LPS dose. However, in a different laboratory, this same *Mpp13* null mouse line was more extensively backcrossed, lost the inactivating *Casp11* passenger mutation, and was no longer protected against lethal LPS injection. This example graphically illustrates how inconsistent phenotypes might arise in different laboratories purportedly using the same congenic mouse line.

In addition to congenic mouse strains, passenger mutations can affect analyses of closely related inbred strains. For example, the IMPC utilizes C57BL/6NJ-derived ESCs to generate novel strains of genetically ablated C57BL/6 mice, and the resulting closely related animals have been presumed to be untainted by genomic background effects. However, Vanden Berghe et al. (2015) identified about 40 nonsynonymous substitutions when they compared the genomes of C57BL/6NJ and C57BL/6 mice. Tellingly, C57BL/6NJ mice are much better protected against lethal LPS injection than are C57BL/6 mice, such that the presence of passenger mutations literally may account for the difference between life and death.

From their bioinformatics analysis, Vanden Berghe et al. (2015) developed a Me-PaMuFind web-based tool that allows researchers to instantly and conveniently identify 129-specific passenger mutations in novel and published gene-targeted mouse strains. Users can employ either the gene symbol or the gene database accession number in order to start their on-line search. The number of backcrosses can be specified, and the quality and quantity of potential passenger mutations are listed at the push of a button. In addition, the program provides the user with PubMed links to the genes in question, as well as direct access to the inventory of genetically modified mouse lines established by the MGI.

The vast majority of existing and well-characterized genetically engineered congenic mouse strains have been created utilizing 129 ESCs followed by extensive backcrosses to the B6 strain. Practicality dictates that these animals will most likely persist in future research endeavors. Thus, awareness of the potential effects of 129-specific passenger mutations and disclosure of their presence in a given experimental mouse model have become scientific necessities. Fortunately, alternative strategies circumventing the use of 129 ESCs to generate genetically modified mutants are now available. Culture conditions for B6 ESCs have been greatly improved over the past couple of years (Gertsenstein et al., 2010; Lin et al., 2014), making it easier to preserve the B6 genetic background. Moreover, Vanden Berghe et al.'s cautionary tale turns a glaring spotlight on the choice of ESC type to be modified using the game-changing CRISPR/Cas genome engineering technology (Ledford, 2015). The wake-up call represented by this *Immunity* article on the importance of the passenger genome therefore does a great public service to the research community involved in the analysis and generation of gene-targeted mice.

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